AD-A169 288 CARBAMATE INDUCED PERFORMANCE DECREMENT RESTORED WITH DIAZEPAM AND ATROPI..(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA C B MATTHEW ET AL. 1/1

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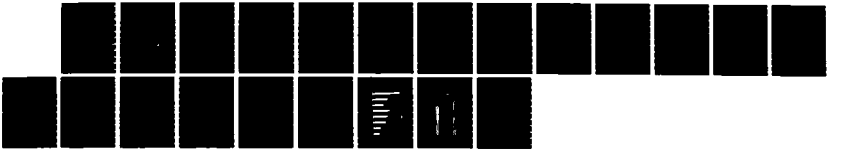

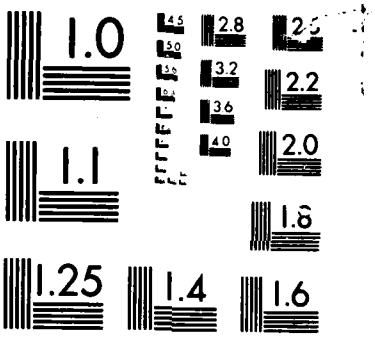
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When rats (500g, male) are exercised to exhaustion on a treadmill, pretreatment with the carbamate physostigmine reduces endurance capacity. Our objective was to determine whether pharmacological intervention could reverse these decrements in performance. The following drugs were administered separately via tail vein: vehicle-control (C), atropine (200 ug/kg, A), diazepam (500 ug/kg, D), and physostigmine (200 ug/kg, PH). After drug administration, rats were run (11 m/min, 6° elevation, Ta = 26°C) to exhaustion (unable to right themselves when placed on their backs). PH administration resulted in reduced

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endurance (41 min PH vs 53 min C, $p<.05$) with greater increments in core temperature (0.090°C/min PH vs 0.057°C/min C, $p<.01$) than control rats. However, when A andD were also given to PH treated rats, the run time and heating rate were restored to control levels. Further, A and D without PH improved performance (82 min, 0.047°C/min) over control levels. Serial administration of an anticholinergic, and anticonvulsant, and an anticholinesterase resulted in no significant change in performance from control levels.

Title: CARBAMATE INDUCED PERFORMANCE DECREMENT RESTORED WITH DIAZEPAM AND ATROPINE IN RATS

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Running head: Carbamate, diazepam, and atropine in running rats

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ABSTRACT

When rats (500g, male) are exercised to exhaustion on a treadmill, pretreatment with the carbamate physostigmine reduces endurance capacity. Our objective was to determine whether pharmacological intervention could reverse these decrements in performance. The following drugs were administered separately via tail vein: vehicle-control (C), atropine (200 ug/kg, A), diazepam (500 ug/kg, D), and physostigmine (200 ug/kg, PH). After drug administration, rats were run (11 m/min, 6° elevation, Ta = 26°C) to exhaustion (unable to right themselves when placed on their backs). PH administration resulted in reduced endurance (41 min PH vs 53 min C, $p<.05$) with greater increments in core temperature (0.090°C/min PH vs 0.057°C/min C, $p<.01$) than control rats. However, when A and D were also given to PH treated rats, the run time and heating rate were restored to control levels. Further, A and D without PH improved performance (82 min, 0.047°C/min) over control levels. Serial administration of an anticholinergic, an anticonvulsant, and an anticholinesterase resulted in no significant change in performance from control levels.

key words: temperature regulation, exercise, anticholinergic, anticholinesterase, heat stress.

Anticholinergic and anticholinesterase drugs used therapeutically or prophylactically for organophosphate poisoning may have undesirable side effects (5). For several years we have been primarily interested in the effects of these drugs on the physical, physiological, and thermoregulatory responses to heat and exercise.

Atropine, the prototype of anticholinergic drugs (5), inhibits evaporative cooling in man by suppressing sweat production (3) and in rats by suppressing saliva production which is behaviorally spread for evaporative cooling (6). Hubbard et al (8) compared the effects of restraint, surgical desalivation, and chemical desalivation with atropine on the ability of rats to thermoregulate in the heat and reported that atropine inhibited thermoregulation to the same extent as surgical desalivation combined with physical restraint. We then used the heat-stressed rat (16) to determine the dose-response effects of atropine, and demonstrated that the rate of rise of core temperature (heating rate) of the rat was the most sensitive index of anticholinergic activity. Therefore, we used the relative heating rates of other anticholinergic drugs to determine a potency for these drugs relative to atropine, and also quantitated the relative ability of various carbamates to reverse the atropine induced increase in heating rate as a measure of anticholinesterase potency (15). Salivation as well as sweating are under muscarinic cholinergic control. In the present study we have extended our model to include nicotinic effects of anticholinergic and anticholinesterase drugs in the exercising rat. Previous work from this laboratory has

established the running rat as a model for human exercise-induced heat injury (9,10).

Physostigmine (PH) was chosen as the representative anticholinesterase because of its efficacy against anticholinergic syndrome (1), and its use with atropine (A) as a prophylaxis against organophosphate poisoning (7). Atropine was chosen to counteract the muscarinic side effects of PH, while diazepam (D) was selected to limit the nicotinic side effects of PH (5). Diazepam has also been used with atropine in the treatment of organophosphate intoxication (24), and PH has been used to reverse the effects of excessive D in both man (14) and rats (20). Thus, our objective was to quantitate the performance decrement induced by the administration of PH or A in the running rat, and to attempt to restore this decrement by pharmacological intervention.

MATERIALS AND METHODS

Experimental Animals: Eight groups of 10 adult male Sprague-Dawley rats (Charles River, CD strain, 510-530 g) were used one time only in all studies. The animals were caged individually in wire-bottomed cages and housed in an environmental chamber (4 x 3 x 2 m) maintained at 26°C and 50% rh. Lighting was controlled automatically (on, 0600-1800 h) and Purina rat chow and water were available ad lib except during experimental intervals.

Drugs: Prior to running, each rat received 3 separate injections 10 min apart via a lateral tail vein. The drugs, doses, and order of administration for each of the 8 groups are presented in Table 1. Atropine (A, 200 ug/kg, as the sulfate, Sigma Chemical Co.) was dissolved in 0.2 ml of sterile 0.9% saline; diazepam (D, 500ug/kg, Valium^{R}, Hoffmann-LaRoche Inc.) was diluted to

0.5 ml with fresh rat serum; and physostigmine (PH, 200ug/kg, Antilirium, Forest Pharmaceuticals) was diluted to 0.02 ml with saline. The order of drug administration (Table 1) was selected (A first and PH last) because A was expected to have the longest duration of action while PH the shortest. Each drug dose used is within the human clinical range for the respective drug when the formula of Freireich et al (4) is used.

Experimental Procedure: Fifteen min after the final (3rd) injection the rats were weighed and then fitted with thermocouples to measure core temperature (Tc, 6.5 cm insertion) and tail skin temperature (Tt, midlength, dorsum); then they were placed on the treadmill. The rats were run at 11 m/min and 6^{o} incline at an ambient of 26^{o}C and 50% rh until they were exhausted (unable to right themselves when placed on their backs). At exhaustion the animals were removed from the treadmill and allowed to recover. During the run and recovery Tc and Tt were monitored and the shocker on the treadmill was controlled by a HP9825 computer-controlled data aquisition system (17).

Statistical Analysis: The data were analyzed by a one way analysis of variance followed by Tukey's test for all pair comparisons, or by Student's unpaired "t" test (Fig. 2). The null hypothesis was rejected at $p<.05$.

RESULTS

Fig.1 illustrates that endurance as measured by run time to exhaustion is inversely correlated with heating rate (rate of increase in core temperature). The figure clearly demonstrates that the PH (cholinesterase= 60% of control) group had the shortest run time and the highest heating rate of the 8 groups.

This elevated heating rate can not be attributed to the tremors observed in this group, because at the start of run (15 min after the last injection) by which time the tremors were subsiding, Tc was lower than that of control rats (Table 2). The A+PH group had a mean endurance time and heating rate that were not significantly different from those of controls; however, the A+PH group did exhibit tremors which were abolished by diazepam in the A+D+PH group. The combination of A+D significantly ($p<.05$) improved endurance over controls and all groups receiving PH.

Weight (wt) loss during the treadmill run (Table 2) is the sum of wt lost through urination, defecation, and salivation (respiratory water loss is negligible). Total % water loss is also a function of the run time which ranged from 41 min for the PH group to 82 min for the A+D group (Fig 1). Atropine decreases both fecal and salivary water loss (8) which explains the lower wt loss rate (Table 2) in the A and A+D groups. Administration of the anticholinesterase PH, which stimulates both salivation and defecation (5), neutralized the A effect on wt loss in the A+PH and A+D+PH groups.

The restraint and multiple injection procedures raised the mean Tc of C rats from 37.5 $\pm$ 0.1°C preinjection to 38.7 $\pm$ 0.2°C at SOR (start of run, 15 min after the 3rd injection). There was a distinct division of the groups (Table 2) into those with high SOR Tc (C, A, D, A+PH) and those with lower SOR Tc (A+D, PH, D+PH, A+D+PH). Except for the A group whose mean Tc EOR (end of run) was significantly higher than that for all other groups, there was no significant difference among the mean Tc's at EOR.

Tail temperature (Tt) at SOR (Table 2) for the first 4 groups are just above the 26°C ambient, but it is interesting to note that the Tt SOR of all groups receiving PH were consistently and significantly higher. Fig. 2

illustrates a potential inverse relationship between the ability to increase tail temperature and the heating rate of the rat. The PH group with the highest heating rate had the smallest increase in Tt while the A+D group had both the lowest heating rate and the largest increment in Tt.

DISCUSSION

As indicated in Fig 1, the heating rate of the running rat was a sensitive index of drug effect. We have previously reported that heating rate is a sensitive index of drug activity in a sedentary heat-stressed rat model (16). The lowest heating rates and longest endurance times were in the D and A+D groups, suggesting that D may have a beneficial effect on thermoregulation during exercise. This hypothesis is supported by the work of Vidal et al (25) who have shown that in the rat hyperthermia induced by handling stress is reversed by diazepam. Also, successive febrile convulsions in infants can be prevented by diazepam administration (11). The doses of D used by Vidal (25) to obtain a reversal of restraint- or injection-induced hyperthermia were higher than the 500 ug/kg used in the current experiments. We have also observed that a dosage of 1.87 mg/kg of D significantly reduced Tc SOR below control levels thus indicating that the hyperthermia induced by handling may be abolished with D; however, the advantageous effects of D on running performance were achieved by the lower dose (500 ug/kg) without effects on Tc SOR or behavior when compared with control groups.

Heat loss through the tail is a major source of heat dissipation in the rat (18, 22, 23). The lower Tc SOR of the PH, D+PH, and A+D+PH groups as well as the ability of A to block this lower Tc SOR in the A+PH group are predicted

by the data of Meeter and Wolthuis (18, 19). These investigators demonstrated that centrally acting anticholinesterases (such as physostigmine) lower Tc by increasing heat dissipation through the tail. Thus, the lower Tc SOR of all groups receiving PH was consistent with their higher Tt SOR (Table 2).

Since a running rat is unable to spread saliva for evaporative cooling, heat dissipation through the tail becomes even more important than in a sedentary animal. Voicu et al (26) demonstrated that the lower Tc of PH-treated rats persists for up to 2 h; however, in exercising rats the blood flow to the working muscles increases at the expense of peripheral blood flow (2). When the rats started exercising, the Tt of PH rats declined and increased only after the rats had become hyperthermic. Fig. 2 illustrates the Tt SOR and EOR for the two groups with the minimal (A+D, 0.047^{o}C/min) and maximal (PH, 0.090^{o}C/min) heating rates. The A+D group increased its Tt by approximately 6^{o}C from SOR to EOR whereas the PH group only increased its Tt by 1.2^{o}C. Tt SOR for the PH group was higher than that for the A+D group, but during exercise the shift of blood to the working muscles proved to be a thermoregulatory liability for the PH group.

Except for the A group there were no significant differences among the Tc EOR for any of the groups. The similar Tc at exhaustion may be explained by the observations of Kozlowski et al (13) whose exercising dogs exhausted after 57 $\pm$ 8 min with core temperatures of 41.8 $\pm$ 0.2 ^{o}C which is very similar to the 53 $\pm$ 4 min and 41.6 $\pm$ 0.2 ^{o}C for the C rats. Their data demonstrated that the muscle content of lactate and the temperature of the working muscle were positively correlated and suggested that hyperthermia induced by exercise causes a change in the metabolism of the working muscles which may limit endurance.

The Tt in the A group was the lowest of all the groups at SOR and throughout the observation period. "Atropine flush" (cutaneous vasodilation induced by atropine administration) has been shown by Kolka et al (12) to cause in humans a cutaneous vasodilation with increased blood flow, increased skin temperature, and increased conductive heat loss in the forearm. However, according to O'Leary et al (21) the neural control of blood flow to the rat tail is analogous to that for apical areas in humans and not the forearm. Therefore, our observation of a lower Tt in the atropinized rat does not necessarily imply that the rat does not exhibit an "atropine flush", but that it may not occur in the tail.

In summary, administration of the anticholinesterase physostigmine to running rats resulted in reduced endurance and an increased rate of rise in core temperature. The performance decrement and elevated heating rate were both restored to control levels by pretreating the animals with the anticholinergic atropine and the anticonvulsant diazepam. Additionally, diazepam, with or without atropine, seems to improve endurance and thermoregulation in the exercising rat. Further research is required to elucidate the mechanism of this improved performance.

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The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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Table 1. Drugs, doses and order of administration

Drugs

C vehicle control - 0.2 ml saline + 0.5 ml serum + 0.2 ml saline

A atropine - 200 ug/kg in 0.2 ml saline

D diazepam - 500 ug/kg in 0.5 ml serum

PH physostigmine salicylate - 200 ug/kg in 0.2 ml saline

GROUP	INJECTIONS*		
	1st	2nd	3rd
C	saline	serum	saline
A	A	serum	saline
D	saline	D	saline
A+D	A	D	saline
PH	saline	serum	PH
A+PH	A	serum	PH
D+PH	saline	D	PH
A+D+PH	A	D	PH

* 10 min apart, via lateral tail vein

TABLE 2. Weight loss and temperature changes in the running rat

Group (N=10)	% Wt loss (%)	Wt loss/ min run (g/min)	Tc[a] SOR (°C)	Tc[b] EOR (°C)	Tt[c] SOR (°C)
C	3.0[d] ±0.2	0.31±0.03	38.6±0.2	41.6±0.2	28.5±0.5
A	1.5* ±0.2	0.13* ±0.02	38.7±0.1	42.2* ±0.2	28.2±0.5
D	4.0±0.2	0.29±0.01	38.5±0.1	41.8±0.1	29.5±0.3
A+D	2.6±0.2	0.18* ±0.02	38.1* ±0.1	41.5±0.1	28.6±0.5
PH	2.9±0.2	0.38±0.02	37.8* ±0.1	41.3±0.1	31.8* ±0.3
A+PH	2.7±0.3	0.28±0.02	38.6±0.2	41.8±0.1	32.1* ±0.3
D+PH	3.7±0.3	0.36±0.03	38.1* ±0.2	41.6±0.2	31.6* ±0.4
A+D+PH	2.8±0.2	0.24±0.02	37.9* ±0.1	41.4±0.2	31.6* ±0.4

a Core temperature - start of run

b Core temperature - end of run

c Tail temperature

d Mean ± S.E.

* Significantly different from controls $p<.05$

FIGURE LEGENDS

Fig. 1 Run time and heating rate for each of the drug treatment groups (see Table 1 for a key to the groups). Values are mean $\pm$ SE, * indicates a significant difference ($p<.05$) from the PH group.

Fig. 2 Tail temperature and heating rates for the PH and A+D groups. Values are mean $\pm$ SE, * indicates a significant difference ($p<.05$) between the 2 groups.

Fig 1

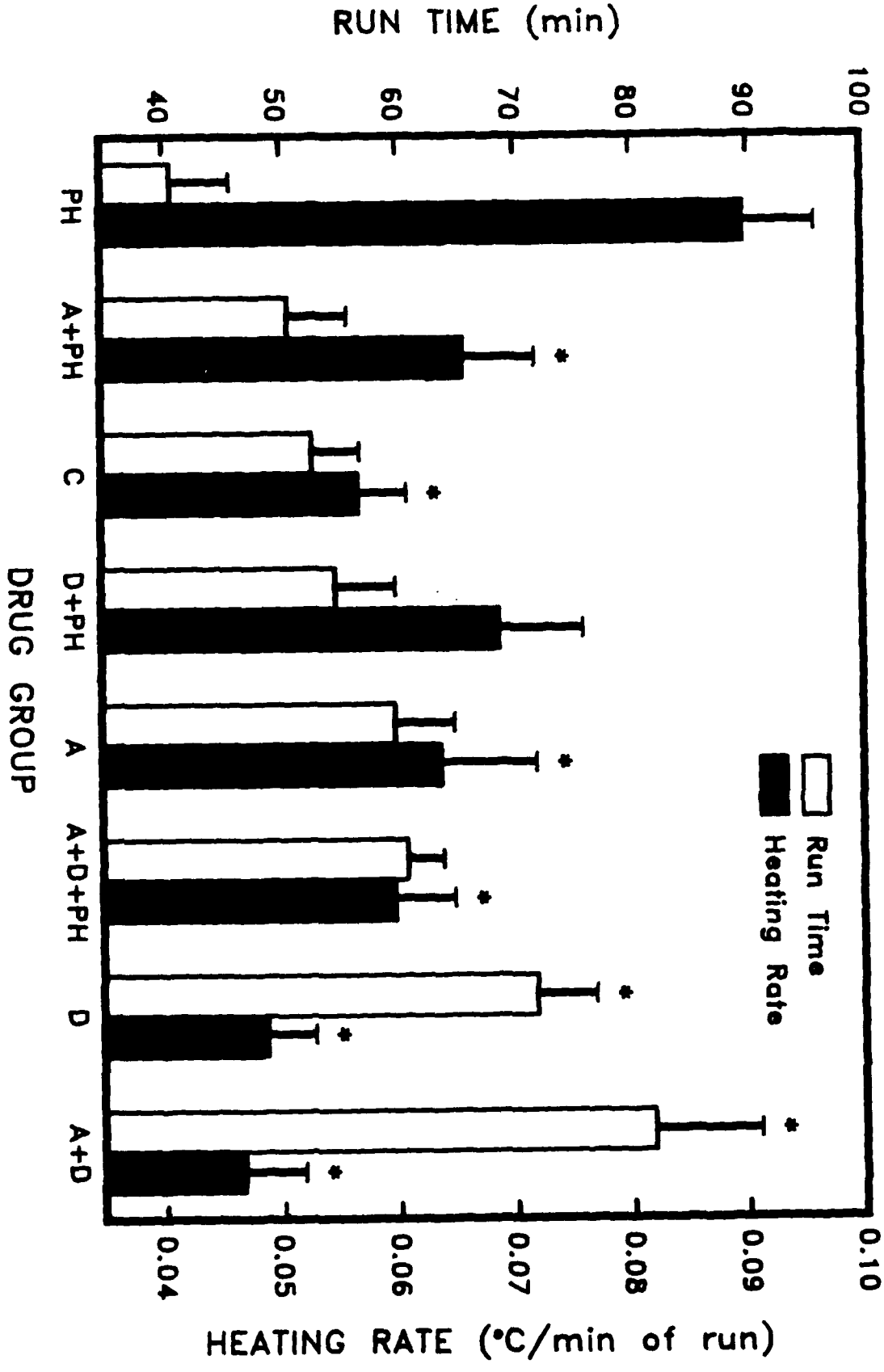
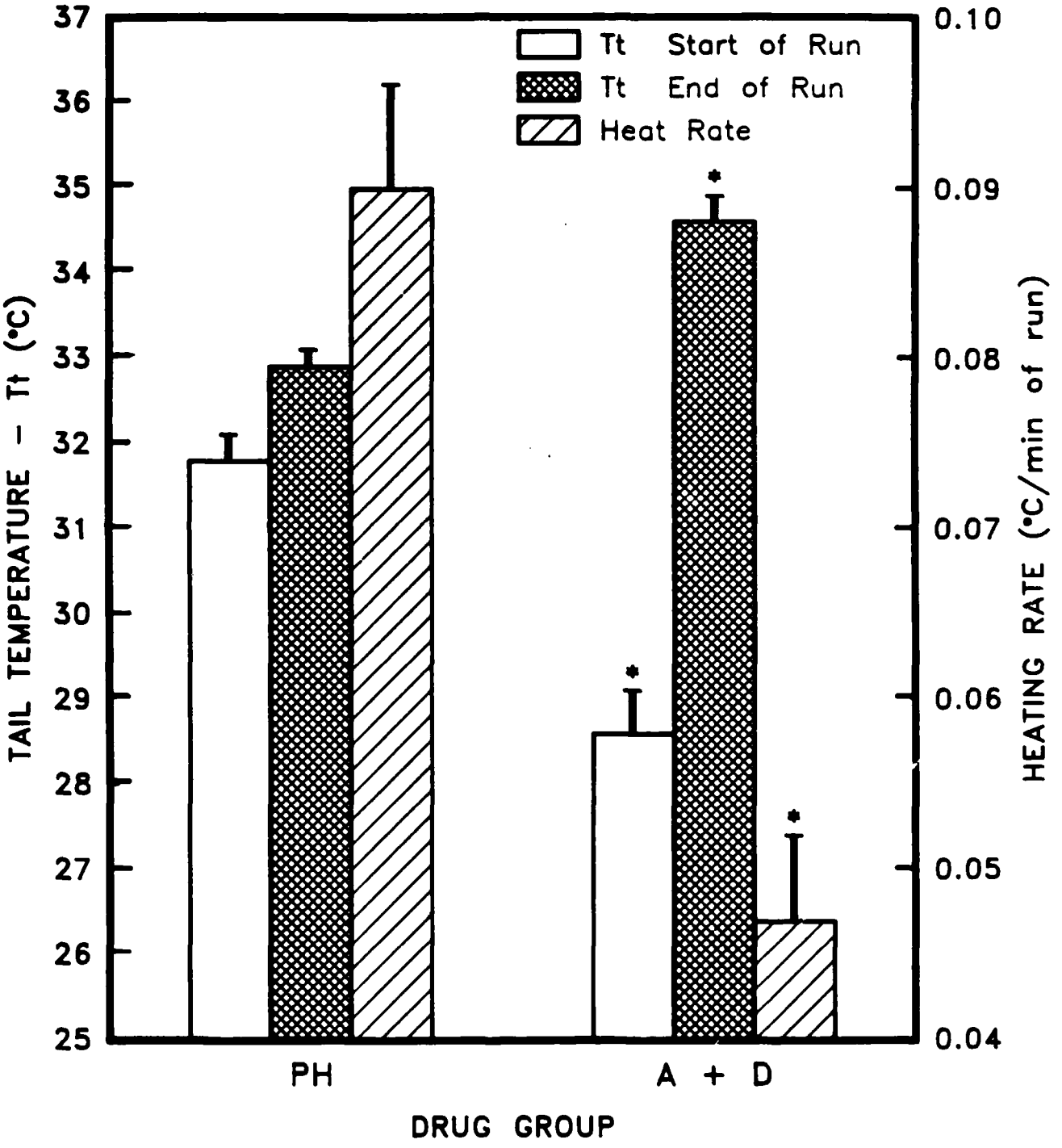

RUN TIME (min)
40
50
60
70
80
90
100
PH
A+PH
C
D+PH
A
A+D+PH
D
A+D
DRUG GROUP
Run Time
Heating Rate
0.04
0.05
0.06
0.07
0.08
0.09
0.10
HEATING RATE (°C/min of run)

Fig 2


Tt Start of Run
Tt End of Run
Heat Rate
TAIL TEMPERATURE - Tt (°C)
HEATING RATE (°C/min of run)
37
36
35
34
33
32
31
30
29
28
27
26
25
0.10
0.09
0.08
0.07
0.06
0.05
0.04
*
*
*
PH
A + D
DRUG GROUP

END

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